**1498-Pos Board B342**
**Combining LAURDAN Generalized Polarization, Fluorescence Correlation Spectroscopy and Fluorescence Lifetime Imaging as a Tool in Skin Diagnostics**
**Maria Bloksgaard**[1,2], Jonathan Brewer[1,2], Susanne Mandrup[2], Luis Bagatolli[1,2].
[1]Membrane Biophysics and Biophotonics group/MEMPHYS – Center for Biomembrane Physics, Odense, Denmark, [2]Department of Biochemistry and Molecular Biology, Odense, Denmark.
The outermost layer of the skin tissue, i.e. the stratum corneum (SC), plays a key role in the barrier function of the skin. SC (10 to 20 micrometer thick) is comprised of dead, keratin filled cells residing in a matrix of complex extracellular lipids. Physical related features of skin SC such as the lipid lateral packing in the extracellular matrix, local proton (water) activity, or local diffusion of particular substances are crucial in determining the permeability of skin towards exogenously added compounds (e.g. drugs and cosmetics). In addition, these parameters may be altered in abnormal skin and further on used as biomarkers for detecting skin anomalies. In this work we decided to map and compare proton activity, polarity (that in turn is related to lipid packing of skin SC membranes) and local diffusion of particular substances in excised skin samples, obtained from wild type mice and abnormal mice, showing increased transepidermal water loss. The experimental techniques utilized are based in multiphoton excitation microscopy techniques. LAURDAN Generalized Polarization (GP) measurements are used to evaluate the overall polarity of potential transepidermal pathways (1). Our results show markedly higher GP values in skin lipid membranes from mice with increased transepidermal water loss compared to controls. To further assess the properties of the skin lipid membranes at the two different physiological conditions, local diffusion through the barrier is evaluated by fluorescence correlation spectroscopy. The data obtained are related to the proton gradient across the SC, as observed by fluorescent lifetime imaging (2). This combined strategy is being tested for potential implementations in skin diagnostics.
1) D.C. Carrer, et al, Journal of Controlled Release (2008), doi:10.1016/j.jconrel.2008.08.006.
2) Hanson et al. 2002, Biophys. J. 83, pp. 1682-1690.

**1499-Pos Board B343**
**Selective Detection of NAD(P)H-Dependent Enzymes during Their Function within Vital Cells by means of Time-Resolved TPLSM**
**Jan L. Rinnenthal**[1], Priyanka Narang[2], Frauke Zipp[1], Raluca Niesner[1].
[1]Charite University Medicine Berlin, Berlin, Germany, [2]Immunology and Infection Unit, Department of Biology, University of York, York, United Kingdom.
The investigation of physiological phenomena at molecular level within vital cells, for instance metabolic processes, represents a major challenge in life sciences. In this context, measuring methods should be non-invasive for the system under study and should provide high sensitivity and specificity. We demonstrate inhere the power of marker-free, spatially and timely resolved intracellular detection of NAD(P)H-dependent enzymes in the study of phenomena of particular biomedical relevance like phagocytosis or autoimmune reactions. The technique is based on biexponential two-photon laser scanning fluorescence lifetime microscopy (TPLSM FLIM) of the physiologic fluorophore NAD(P)H, which exhibits a fluorescence lifetime $\tau$ that is strongly dependent on its chemical environment, i.e. on the enzyme to which NAD(P)H is bound to (free NAD(P)H: $\tau \sim 400$ ps, enzyme-bound NAD(P)H: $\tau \sim 2$ ns). In order to verify the feasibility of the method, an in-vitro study with different enzymes in solution was performed. The measured fluorescence lifetimes of enzyme-bound NAD(P)H strongly varied from $\tau = 960 \pm 27$ ps for malic dehydrogenase to $\tau = 3640 \pm 101$ ps for 3$\alpha$ hydroxysteroid dehydrogenase, which made us confident, that an intracellular selective enzyme detection is possible by means of NAD(P)H-FLIM. We indeed succeeded for the first time to specifically and intracellularly detect the NADPH oxidase, a multi-subunit membrane-bound enzyme complex that catalyzes the reduction of free oxygen to its superoxide anion. Thereby, we selectively monitored the NADPH oxidase during its function within differently activated murine polymorphonuclear leucocytes (PMNs). The experiments revealed a specific fluorescence lifetime of $3670 \pm 140$ ps for NADPH bound to this enzyme both in humorally activated PMNs, i.e. activation with PMA, and in PMNs phagocytosing the fungus Aspergillus fumigatus, confirming the assembly of NADPH oxidase as highly site-specific.

**1500-Pos Board B344**
**Imaging Collagen for Normal and Pathological Skin Dermis through Polarization-Resolved Second Harmonic Generation**
**Ping Jung Su**[1], Wei-Liang Chen[1], Tsung-Hsien Li[1], Ruei-Jr Wu[1], Jin-Bon Hong[2], Vladimir Hovhannisyan[1], Sung-Jan Lin[2], Chen-Yuan Chen[1].
[1]National Taiwan University, Taipei, Taiwan, [2]National Taiwan University Hospital, Taipei, Taiwan.
Polarization-resolved, second harmonic generation (P-SHG) microscopy is utilized for medical diagnosis of pathological skin dermis. We acquired the pitch angle of collagen fiber from the P-SHG microscopy. In analyzing the large area, pixel by pixel of normal and pathological skin dermis, we found that excessive proliferation of collagen fiber (EPC) and dissolution of elastic fiber (DEF) characterize pathological skin dermis. In addition, the pitch angle of normal skin was found to be $52.01 \pm 0.96$, while pathological skin EPC and DEF were found to be $48.68 \pm 1.49$ and $48.09 \pm 1.83$. It is found that pitch angle of pathological dermis trend to smaller angle, below 50 degree, and wider distribution, 2 times over the normal.

**1501-Pos Board B345**
**Intravital Multiphoton Microscopy For Imaging Hepatobiliary Function In Vivo**
**Feng-Chieh Li**, Chen-Yuan Dong.
National Taiwan University, Sec. 4, Roosevelt Rd., Da-an District,, Taiwan.
When liver can not be regenerated in time to amend damages that has occurred, failure of hepatic functions such as liver failure and metabolic disease can result. Traditionally, the study of liver pathology has depended on histological techniques, but such methods are limited to ex-vivo observation. In order to study hepatic metabolism in vivo, we have designed a hepatic imaging chamber made of biocompatible titanium alloy (6V4Al-Ti). In combination with multiphoton and a method of quantification by using optical signal, our approach allows the intravital observation of hepatic activities to be achieved. Processes such as hepatic metabolism and disease progression can be studied using this methodology.

**1502-Pos Board B346**
**Second Harmonic Generation Microscopy Characterization of Corneal Edema**
**Chiu-Mei Hsueh**[1], Wen Lo[1], Hsin-Yuan Tan[2], Chen-Yuan Dong[1].
[1]National Taiwan University, Taipei, Taiwan, [2]Department of Ophthalmology, Chang Gung Memorial Hospital, College of Medicine, Chang Gung University, Linko 333, Taiwan, Linko, Taiwan.
The purpose of this study is to investigate the structural features of corneal edema by multiphoton fluorescence and second harmonic generation (SHG) microscopy and the potential of this technique as a clinical, *in vivo* monitoring technique for cornea disease diagnosis. Corneal edema is a build up of fluid or swelling of the cornea that can produce clouding of the cornea and significant decrease in vision. Since collagen can be induced to generate strong second harmonic generation (SHG) signal, multiphoton excitation provide direct visualization of collagen orientation within corneal stroma.
In this study, normal and over-hydrated bovine corneal specimens used in this study were observed to distinguish the structural alteration due to edema. We collected forward and backward SHG signals simultaneously within different depths and also with large region images. From the result, we found that the organization of corneal stroma was not significantly altered, except for the increase of interfibrillar spacing. This structural information provided by multiphoton imaging may help the evaluation of the necessity for full-thickness corneal transplantation in pathological cases.

**1503-Pos Board B347**
**New Dyes With Fast Voltage-Dependent Changes In Membrane SHG**
**Stacy A. Wilson**, Aifang Xie, Ping Yan, Leslie M. Loew.
University of Connecticut Health Center, Farmington, CT, USA.
Laser scanning second harmonic generation (SHG) microscopy has shown significant promise for membrane potential imaging with voltage sensitive dyes (VSDs), possessing significant advantages over fluorescence-based imaging modalities. Through simultaneous patch-clamping and non-linear imaging of cells, SHG has been found to exhibit sensitivities to trans-membrane potential that are up to four times better than those obtained under optimal conditions using one-photon fluorescence imaging (Millard *et al.*, 2004). For styryl dyes, while electrochromism is the dominating photophysical mechanism of fluorescence, some ANEP-based dyes display slow SHG voltage responses, suggesting that chromophore membrane reorientation or redistribution may be involved. The mechanism of the SHG response is not entirely understood and necessitates additional study in order to fully optimize this imaging modality. We report on our further investigation of the time dependence of the voltage sensitivity of SHG and simultaneous two-photon fluorescence imaging, using ''fast'' voltage-switching experiments. The response kinetics of resonance enhanced SHG from several styryl dyes developed in our laboratory, including di-3-ANEPPDHQ, as well as di-4-ANEPPTEA and a new fluorinated derivative, have been determined. Voltage-clamped neuroblastoma cells stained with these dyes were imaged with 1064 nm excitation from a mode locked fiber-based